

# Use of Endogenous, Stable Lead Isotopes to Determine Release of Lead from the Skeleton

Donald R. Smith,<sup>1,2</sup> John D. Osterloh,<sup>2</sup> and A. Russell Flegal<sup>1</sup>

<sup>1</sup>Environmental Toxicology, University of California, Santa Cruz, CA 95064 USA; <sup>2</sup>Laboratory Medicine, University of California, San Francisco, CA 94110 USA

The stable lead isotope methodology can be used to study the release of lead from bone into the circulation because of its potential to distinguish circulatory lead from "older" and isotopically different skeletal lead that may have been accumulated years or decades earlier. Here we report the initial results from a larger ongoing study that evaluates the skeleton as a source of lead to the circulation in environmentally exposed human subjects. Lead concentrations and stable lead isotopic compositions were measured in blood and trabecular bone samples obtained from five patients who underwent total hip or knee joint replacement. All subjects contained low blood (1–6 µg/dl) and bone (0.6–7 µg/g dry weight) lead concentrations typical of environmentally exposed individuals. There were relatively large differences in the lead isotopic compositions between the paired blood and bone samples from each subject. These isotopic differences are attributed to differences in the lead isotopic compositions of past versus current lead exposures and to the long elimination half-life of lead in the skeleton compared to lead in the circulation. Based on these data, we determined that the skeleton contributed 40–70% of the lead in the blood of these subjects. This initial study demonstrates the utility of the stable lead isotope methodology for investigating the release of lead from the skeleton. It also shows that the skeleton can be an important endogenous source of lead exposure in environmentally exposed humans. **Key words:** lead, skeleton, stable isotopes, tracers. *Environ Health Perspect* 104:60–66(1996)

Historically regarded as an inert sink for lead, the skeleton is now recognized to be as important in the kinetic behavior of lead as are the influences of exposure, absorption, and elimination (1,2). The skeleton is the predominant (>90%) endogenous storage site for lead (3–5). The bulk of that lead is contained within long-lived compartments of cortical [elimination half-life ( $t_{1/2}$ ) >5–10 years] and trabecular (elimination  $t_{1/2}$  >1 year) bone, with comparatively small amounts of lead in tissue compartments that rapidly exchange with extracellular fluid and plasma (1,2,6–8). Because lead is qualitatively a biologic analog to calcium, its uptake and release from the skeleton are, in part, controlled by many of the mechanisms that regulate calcium and bone mineral homeostasis (1–13). These include normal mineral diffusion and turnover (apposition/resorption) processes (1,2,9–13).

Clinical and experimental studies have provided evidence that the skeleton may serve as an endogenous source of lead to the circulation. This has been suggested to occur during normal homeostasis (14,15), after the cessation of chronic occupational exposures (8,16), during periods of accelerated bone turnover and mineral loss, such as in osteoporosis, pregnancy, and lactation (14,17–19), and under conditions of thyroid and parathyroid hormone imbalances (20,21). Nonetheless, relatively little is known about the extent to which lead is mobilized out of bone in environmentally

exposed individuals (22,23), and the information that does exist is largely indirect.

The use of stable lead isotope ratios as a tracer methodology for evaluating lead exposure and metabolism is based on differences in the relative amounts of the four naturally occurring stable isotopes of lead: <sup>204</sup>Pb, <sup>206</sup>Pb, <sup>207</sup>Pb, and <sup>208</sup>Pb. The natural relative abundances of these isotopes vary between different geologic sources of lead, and these isotopic differences persist when the lead is mined and incorporated into industrial materials (e.g., gasoline, paints). Typically, industrial leads contain a mixture of leads from different geologic sources as well as recycled lead. Since the geologic sources of lead used in industrial materials has changed over the past several decades, there have also been temporal changes in the isotopic compositions of contaminant leads discharged to the environment (24–28).

For example, the <sup>207</sup>Pb/<sup>206</sup>Pb ratios in industrial lead produced and used in the United States ranged from about 0.862 to 0.983 prior to the late 1960s, since much of the lead mined at that time came from geologically older lead ores with relatively low amounts of <sup>206</sup>Pb (27). The mean ratio in U.S. leads then began to change because of the use of younger lead ores from Missouri, which possessed greater amounts of <sup>206</sup>Pb and lower <sup>207</sup>Pb/<sup>206</sup>Pb ratios (e.g., 0.741) (27). In addition, recycled lead metal contributed substantially to

domestic lead production by the late 1960s, which also had an impact on the isotopic compositions of contaminant leads discharged to the environment. As a result, the isotopic compositions of human lead exposures have changed temporally in the United States and elsewhere, although it is difficult to reconstruct those changes in detail due to the limited amount of available data.

Stable lead isotope methodologies are well suited for studying skeletal lead, which may be distinct from lead in other tissues (29,30). This is facilitated by the long residence times of lead in the mammalian skeleton, which greatly exceed those in the soft tissues, and by the temporal changes in the isotopic compositions of industrial lead emissions to the human environment. As a result, stable lead isotopes may be used to distinguish circulatory lead derived from current environmental exposures from "older" skeletal lead that was assimilated into bone over past years or decades (6,14,27,29,30).

Here we report the initial results from a larger ongoing study to evaluate the skeleton as a source of lead to the blood circulation in environmentally exposed human subjects. In this study, lead concentrations and stable lead isotopic compositions were measured in blood and bone samples from patients undergoing total hip or knee joint replacement. These initial data will be used to illustrate both the advantages and limitations of this methodology for assessing lead exposure to humans from multiple sources. A complete report and discussion of our results on the extent of skeletal lead release for the entire cohort of the larger study will be presented in a subsequent paper when those data become available.

Address correspondence to D. R. Smith, Environmental Toxicology, Applied Sciences Building, Room 254A, University of California, Santa Cruz, CA 95064 USA.

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## Methods

### Stable Lead Isotope Ratio Methodology

The stable lead isotope methodology for distinguishing multiple sources of lead to the circulation is based on the occurrence of different and measurably distinguishable lead isotopic compositions of the various sources. Because this methodology measures all four stable isotopes ( $^{204}\text{Pb}$ ,  $^{206}\text{Pb}$ ,  $^{207}\text{Pb}$ , and  $^{208}\text{Pb}$ ) within a sample, it may be possible to assign isotope ratio signatures as “source-specific” tracers of different sources of lead exposure. For the purposes of this article, it is assumed that lead inputs to the circulation in humans can be simply represented by sources of internal (endogenous) and external (exogenous) origin. We further assumed that the skeleton is the main endogenous source of lead, while the principal exogenous sources of lead exposure in this cohort are from diet, dust, and water (i.e., ingestion and inhalation). This two-source model of lead inputs to the circulation is a simplification of what in some cases may be a complex system with multiple exogenous and endogenous inputs.

Differences in lead isotopic compositions may occur between the skeletal and circulatory lead pools in humans because of the long residence times of lead in the skeleton and the temporal changes in the isotopic compositions of industrial lead emissions to the environment (8,14,25–27,29). The skeleton contains lead accumulated over approximately the past 20 years (“older” and often isotopically different lead), whereas the blood reflects a mixture of recent exposures and any endogenous bone contribution. Lead isotopic differences between blood and bone may be used to evaluate the release of lead from the skeleton, providing that 1) the isotopic compositions of the primary sources of lead to the circulation (i.e., endogenous and exogenous) can be characterized or estimated, and 2) the endogenous and the primary exogenous sources of lead exposure have measurably different lead isotopic compositions.

Under these conditions, the amount of lead in the circulation originating from skeletal versus exogenous sources can be calculated using three isotope linear regression analyses (i.e., using ratios with a common denominator such as  $^{207}\text{Pb}/^{206}\text{Pb}$  versus  $^{208}\text{Pb}/^{206}\text{Pb}$ ) (29–31). The isotopically distinguishable lead in the skeleton and in the exogenous sources of exposure represent the two sources of lead input to the circulation. When the blood is receiving input predominantly from these two sources, its isotopic composition will fall along a “mixing line” defined by those

sources, and the linear distance along that mixing line will represent the relative contribution of lead from each source to the lead in blood (Fig. 1).

The relative contribution from each source (skeletal versus exogenous) to the lead in the circulation can be calculated using the following formula:

% Skeletal lead in blood =

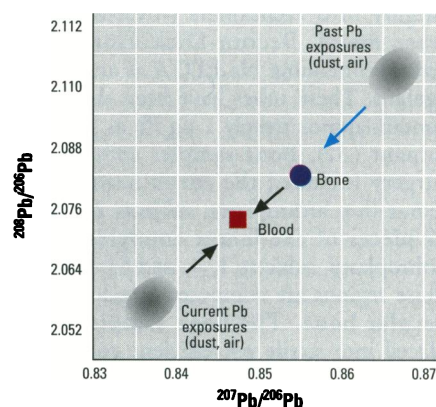
$$\frac{\left( \frac{^{207}\text{Pb}}{^{206}\text{Pb}} \right)_{\text{bl}} - \left( \frac{^{207}\text{Pb}}{^{206}\text{Pb}} \right)_{\text{ex}}}{\left( \frac{^{207}\text{Pb}}{^{206}\text{Pb}} \right)_{\text{bn}} - \left( \frac{^{207}\text{Pb}}{^{206}\text{Pb}} \right)_{\text{ex}}} \times 100$$

where  $(^{207}\text{Pb}/^{206}\text{Pb})_{\text{bl}}$  = isotope ratio in blood;  $(^{207}\text{Pb}/^{206}\text{Pb})_{\text{ex}}$  = isotope ratio of the exogenous exposures; and  $(^{207}\text{Pb}/^{206}\text{Pb})_{\text{bn}}$  = isotope ratio in bone. Although the  $^{207}\text{Pb}/^{206}\text{Pb}$  ratios were used in this example, other ratios such as  $^{208}\text{Pb}/^{206}\text{Pb}$  can also be used, as long as the three-isotope regression is consistent with the presence of a two-source system. If only two sources exist, then using either of the ratios (e.g.,  $^{207}\text{Pb}/^{206}\text{Pb}$  or  $^{208}\text{Pb}/^{206}\text{Pb}$ ) will yield the same result.

If there is an additional source of lead that has an isotopic composition different from the two primary sources, the isotopic composition of a measured blood sample will deviate along (or off) the regression line by a distance proportional to the amount and isotopic composition of lead that was derived from that additional source. The same approach can also apply when evaluating how well a suspected lead source (e.g., an estimated exogenous exposure) fits a two-source system defined by a measured source (bone) and a measured sample mixture (blood).

### Subjects

Five subjects (three males, two females, ages 52–75 years) undergoing total hip or knee arthroplasty within the Orthopedic Surgical Unit at San Francisco General Hospital were included in the initial study to assess the utility of the stable isotope methodology for evaluating bone lead release in environmentally exposed humans. These five subjects were among the first individuals recruited for the larger ongoing study, which will contain approximately 20 subjects. Subjects were selected without any *a priori* selection/exclusion criteria other than their need for hip or knee joint replacement. The subjects gave written, informed consent, and the study was approved by the Committee on Human Research at the University of California, San Francisco. A venous whole-



**Figure 1.**  $^{207}\text{Pb}/^{206}\text{Pb}$  versus  $^{208}\text{Pb}/^{206}\text{Pb}$  ratios in paired blood and bone samples from subject 1. Also included are the isotope ratios of estimated current lead exposures and past lead exposures (25). The bone isotopic composition reflects previous inputs from past lead exposures (blue arrow), whereas the blood isotopic composition reflects recent inputs from exogenous (current Pb) and endogenous (bone) sources (black arrows). Differences in isotope ratios between paired blood and bone samples are attributed to the long elimination half-life ( $t_{1/2}$ ) of lead in the skeleton (>1–5 years) compared to lead in the circulation (<30 days), and to the differences in the lead isotopic compositions of current versus past lead exposures.

blood sample was obtained at the time of each subject's presurgery examination. Bone samples were obtained at surgery and were composed of specimens normally removed and discarded with total hip or knee arthroplasty, as described below.

During patient recuperation in the hospital, a questionnaire was administered to collect information on each subject's occupational and residential history over the past 20 years to identify specific sources of lead exposure and to provide a general record of the subject's medical and reproductive history. One of the subjects (#1) had lived in the San Francisco area >20 years; three (#2, 3, and 4) had lived in the San Francisco area 12–15 years (previously residing in Nicaragua, Brazil, and the Philippines, respectively); and one subject (#5) had lived in the San Francisco area for only 2 years (previously residing in Nigeria). The predominance of foreign-born subjects in this initial study reflects the ethnic make-up of the patient population at San Francisco General Hospital. There was no indication of excess lead exposure for any of the subjects, based on their reports and occupations over the past 20 years. Thus, their general exposure patterns are typical low-level environmental lead exposures.

### Sample Collection

We collected venous whole-blood samples (5 to 7 ml) approximately 5–10 days before

surgery into vacutainers (royal-blue-top trace metal, Becton-Dickinson, New Jersey) containing Na<sub>2</sub>EDTA as an anticoagulant. These tubes have been shown to contain approximately 1 ng Pb as a contaminant (32). Bone samples collected at surgery included the entire head of the femur (hip arthroplasty, subjects 2 and 3) or pieces of the femur condyles (knee arthroplasty, subjects 1, 4, and 5). Both of those regions are composed largely of trabecular bone. The bone samples were placed into sterile polyethylene containers at collection. All bone and blood samples were kept frozen until analyses.

## Analyses

We conducted all sample processing and analyses in a trace-metal-clean HEPA-filtered air (class 100) laboratory using ultra-clean trace-metal techniques (29). These techniques are required for clinically or environmentally based stable lead isotope tracer studies to avoid sample contamination and invalidation of the results (24,29,33,34). Laboratory ware (Teflon, polyethylene, and polypropylene) and the titanium and stainless-steel bone-sampling instruments were acid-cleaned using procedures detailed by Flegal and Smith (33). Reagents were double sub-boiling quartz distilled, and water was high-purity grade (18 MΩ-cm).

Aliquots (0.5 ml) of blood samples were transferred to Teflon vials, weighed, and oven dried before acid digestion. We removed aliquots of bone from the head or condyles of the femur using a custom-fabricated, hollow titanium alloy drill bit (5.4 mm internal diameter). Bone-core aliquots were obtained by drilling through the center of the femur head (anterior-posterior axis) or piece of condyle. There was no visible sign of bone degeneration in any of the samples selected for analyses. Retrieved aliquots of bone were sequentially trimmed with a stainless-steel scalpel and rinsed repeatedly with 1% quartz-distilled HNO<sub>3</sub> and ultrapure water to obtain a final analytical sample from within the bone core. Analytical aliquots of bone were transferred to Teflon vials and oven dried (65°C for 3 days) to a constant weight. Based on previous experience, as well as concurrent experiments to evaluate lead contamination, this bone sampling procedure yielded an uncontaminated aliquot composed largely of trabecular bone.

Dried blood and bone samples were digested in hot 16 N HNO<sub>3</sub> for about 8 hr, evaporated to dryness, and redissolved in 1 N HNO<sub>3</sub> (29). We measured lead concentrations in sample aliquots by graphite furnace atomic absorption spectrometry (GFAAS), using the method of

additions to minimize matrix interferences (29). Procedural accuracy of the lead concentration measurements was quantified by concurrent analyses of National Institutes of Standards and Technology Standard Reference Material 955a (blood). The analytical limit of quantitation of lead by GFAAS was 0.02 µg/dl.

After measuring lead concentrations, we processed aliquots of digested samples for lead isotopic composition analyses using an anion-exchange resin separation technique (29). Lead isotopic compositions were measured by thermal ionization mass spectrometry (TIMS) using a VG Sector 354 multicollector instrument. Total cumulative lead (mean ± coefficient of variation) in procedural (processing and analyses) blanks was determined to be 60 pg ± 30% by isotope dilution TIMS. This blank amount was <1% of the lead content of the lowest lead sample aliquot analyzed. Blank corrections of the isotopic ratios and the analyses of propagated errors were calculated using methods described previously (29). The final propagated error of the isotope ratio measurements was ≤0.02% relative standard error (RSE).

We determined the relative amount (percent) of lead in the circulation that was derived from the skeleton using the formula presented above. The percentage of skeletal lead in blood was calculated using both <sup>207</sup>Pb/<sup>206</sup>Pb and <sup>208</sup>Pb/<sup>206</sup>Pb ratios, and those two results were averaged (mean ± SD). The major isotope ratios, <sup>207</sup>Pb/<sup>206</sup>Pb and <sup>208</sup>Pb/<sup>206</sup>Pb (isotopes of greater natural relative abundance), were used for these calculations, rather than ratios with the minor isotope <sup>204</sup>Pb, because the former can be measured with greater precision and has

proven more useful in distinguishing lead sources (24,29,35).

We hypothesized that the isotopic composition of the current "net" exogenous exposure for these subjects could be characterized by the lead in local particulate aerosols and tap water. Therefore, we qualitatively derived an estimate of this exposure using the isotopic compositions (i.e., <sup>207</sup>Pb/<sup>206</sup>Pb, <sup>208</sup>Pb/<sup>206</sup>Pb, <sup>206</sup>Pb/<sup>204</sup>Pb, <sup>207</sup>Pb/<sup>204</sup>Pb, and <sup>208</sup>Pb/<sup>204</sup>Pb) of previously measured urban aerosols/dusts and tap water samples from the San Francisco area (28,36). Current exposure values extrapolated from the regression lines defined by the subjects' blood-bone pairs were also incorporated into this estimate (see Discussion).

## Results

The levels of lead in the blood (1–6 µg/dl) and bone (0.6–7 µg/g dry weight) of these subjects were relatively low, and levels varied between subjects (Table 1). These data indicate an absence of recent or chronically elevated past lead exposures, which is consistent with the questionnaire results of the subject's residence and occupation history over the past 20 years. Based on current models of lead exposure (37), their principal sources of exposure were from environmental lead, including lead in urban dusts, food, water, and particulate aerosols.

There were relatively large differences between the lead isotopic compositions of the paired blood and bone samples for each of the subjects, with bone exhibiting notably larger <sup>207</sup>Pb/<sup>206</sup>Pb and <sup>208</sup>Pb/<sup>206</sup>Pb ratios in all cases (Table 1; Fig. 2). The isotopic differences between paired blood and bone samples (e.g., 0.4–0.9% for <sup>207</sup>Pb/<sup>206</sup>Pb ratios) were easily discernible with TIMS

**Table 1.** Lead concentrations and stable lead isotopic compositions in paired blood and bone samples and the calculated relative percent of the lead in blood derived from the skeleton

Subject no.	Age/sex	Sample	Pb				% Bone-derived Pb in blood <sup>c</sup>
			concentration <sup>a</sup>	<sup>207</sup> Pb/ <sup>206</sup> Pb <sup>b</sup>	<sup>208</sup> Pb/ <sup>206</sup> Pb	<sup>206</sup> Pb/ <sup>204</sup> Pb	
1	61/M	Blood	3.4	0.8474 (0.0002)	2.073 (0.001)	18.462 (0.010)	61 ± 8
		Bone	6.3	0.8550 (0.0000)	2.082 (0.001)	18.232 (0.007)	
2	52/F	Blood	1.3	0.8400 (0.0000)	2.063 (0.001)	18.646 (0.021)	42 ± 24
		Bone	0.74	0.8460 (0.0000)	2.069 (0.001)	18.526 (0.016)	
3	58/M	Blood	5.7	0.8563 (0.0002)	2.088 (0.001)	18.224 (0.009)	71 ± 1
		Bone	6.9	0.8634 (0.0002)	2.102 (0.001)	18.060 (0.007)	
4	75/M	Blood	3.1	0.8510 (0.0002)	2.074 (0.001)	18.352 (0.011)	72 ± 2
		Bone	1.4	0.8564 (0.0001)	2.081 (0.001)	18.200 (0.009)	
5	69/F	Blood	2.8	0.8648 (0.0002)	2.108 (0.001)	18.012 (0.011)	— <sup>d</sup>
		Bone	0.65	0.8680 (0.0001)	2.117 (0.001)	17.997 (0.011)	

<sup>a</sup>Blood and bone lead concentrations in units of µg Pb/dl blood and µg Pb/g dry bone, respectively.

<sup>b</sup>Isotope ratio errors in parentheses are 2 SEs of measurement by thermal ionization mass spectrometry.

<sup>c</sup>Values are averages (±SD) of results calculated using the <sup>207</sup>Pb/<sup>206</sup>Pb and <sup>208</sup>Pb/<sup>206</sup>Pb ratios (see Methods).

<sup>d</sup>Unable to calculate for this subject because the estimate of current exposure derived for the other four subjects did not appear to adequately represent the predominant source of exogenous exposure to this individual (see Results).

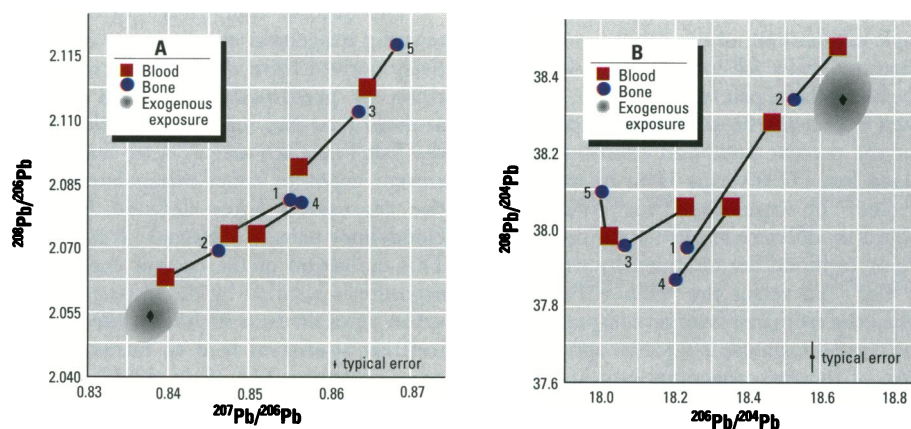


because they exceeded the isotope ratio error of measurement ( $\leq 0.02\%$  RSE) by 20-fold to  $>40$ -fold. There were also substantial differences in the blood and bone isotopic compositions between the subjects.

The differences in isotopic compositions between the blood–bone sample pairs from each subject are attributed to differences in the lead isotopic compositions of current versus past lead exposures and to the long elimination half-life of lead in the skeleton compared to lead in the circulation. This is illustrated in Figure 1 using the data of subject 1. His skeleton appears to contain a relatively large component of “older” or previously accumulated lead, based on its similarity to the isotopic composition of urban dusts/aerosols and gasoline from California (Los Angeles) several decades ago (25). In contrast, the blood isotopic composition of this subject (as well as that of other subjects) more closely resembles current urban leads in the San Francisco area (28,36).

The relative amount of lead in blood that was derived from the skeleton was calculated for each of the subjects using the isotopic compositions measured in their blood and bone samples and an estimated isotopic composition of their current net exogenous exposure of  $^{207}\text{Pb}/^{206}\text{Pb} = 0.8380$ ,  $^{208}\text{Pb}/^{206}\text{Pb} = 2.0545$ , and  $^{206}\text{Pb}/^{204}\text{Pb} = 18.660$ . As mentioned above, this estimate of current exogenous exposure was qualitatively derived using the isotopic compositions of previously measured urban aerosols/dusts and tap water from the San Francisco area (28,36) and current exposure values extrapolated from the regression lines defined by the subjects’ blood–bone pairs. This estimate is included with an uncertainty oval (95% confidence limits) in Figures 1 and 2. The isotopic composition of their current exogenous exposure was determined indirectly because of the limitations and difficulty associated with directly measuring all possible sources of exposure and then evaluating the relative contributions of those sources to the lead assimilated into blood.

Using three-isotope linear regression analyses, the relative amount (percent) of lead in blood that was derived from the skeleton (mean  $\pm$  SD, using both the  $^{207}\text{Pb}/^{206}\text{Pb}$  and  $^{208}\text{Pb}/^{206}\text{Pb}$  ratios) was determined to be  $61\% \pm 8\%$ ,  $42\% \pm 24\%$ ,  $71\% \pm 1\%$ , and  $72\% \pm 2\%$  for subjects 1, 2, 3, and 4, respectively (Table 1). We did not calculate the bone lead contribution to blood for subject 5 (a recent immigrant from Nigeria), since the estimate of current exogenous exposure derived for the other four subjects did not appear to represent the predominant source of exogenous exposure for this individual. This was apparent when



**Figure 2.** (A)  $^{207}\text{Pb}/^{206}\text{Pb}$  versus  $^{208}\text{Pb}/^{206}\text{Pb}$  ratios and (B)  $^{206}\text{Pb}/^{204}\text{Pb}$  versus  $^{208}\text{Pb}/^{204}\text{Pb}$  ratios in paired blood and bone samples from the five subjects (paired blood–bone samples for each subject are connected by a solid line; subject number listed beside each symbol). Also included is the estimated current lead exposure (surrounding oval indicates 95% confidence limits) for subjects 1–4. The estimate of current lead exposure does not adequately represent the predominant source of exogenous exposure to subject 5, based on the  $^{206}\text{Pb}/^{204}\text{Pb}$  and  $^{208}\text{Pb}/^{204}\text{Pb}$  ratios in her blood (i.e., her blood lead isotopic composition is not directionally oriented toward the current lead exposure isotopic composition). This was not evident from the  $^{207}\text{Pb}/^{206}\text{Pb}$  and  $^{208}\text{Pb}/^{206}\text{Pb}$  ratios. The typical errors are 2 SEs of measurement by thermal ionization mass spectrometry.

the  $^{206}\text{Pb}/^{204}\text{Pb}$  and  $^{208}\text{Pb}/^{204}\text{Pb}$  ratios were considered (Fig. 2B) and was not evident from the plot of  $^{207}\text{Pb}/^{206}\text{Pb}$  versus  $^{208}\text{Pb}/^{206}\text{Pb}$  ratios (Fig. 2A). We could not estimate the alternate sources of exogenous exposure for subject 5, who may have dietary or other habits that contribute to a unique lead exposure.

## Discussion

Many urban-dwelling adults aged 25 years and older are likely to have elevated bone stores of lead, due to the lack of regulation of lead sources in the United States and elsewhere before the mid-1970s. Subsequent regulation of several major sources of lead exposure (e.g., lead in industrial emissions, gasoline, paint, and solder) has contributed to declines in blood lead levels in the United States and in several other countries (38,39). Consequently, the importance of elevated burdens of endogenous lead within the skeleton relative to exogenous environmental exposures may be increased in this cohort.

The results of this initial study are consistent with that concern. They indicate that previously accumulated lead stores within the skeletons of these environmentally exposed subjects are serving as important sources of lead by contributing 40–70% of the lead in the circulation. The differences in bone isotopic compositions between the subjects reflect the different sources of past exposures and possibly different rates of exchange of accumulated bone lead with isotopically different lead in the circulation. These data are consistent

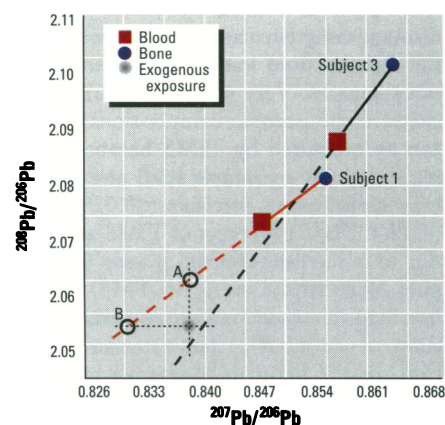
with a study by Manton (14), who used a similar methodology to estimate that the skeleton contributed as much as 70% of the lead in the circulation of an adult male.

This stable isotope methodology offers several important advantages over other currently available methods to evaluate skeletal lead release. It is capable of identifying and distinguishing circulatory lead derived from the skeleton from lead derived from exogenous sources. Very small ( $<0.1\%$ ) differences in the isotopic compositions of lead can be measured by TIMS because of its sensitivity ( $<1$  ng sample lead) and isotope ratio measurement precision ( $\leq 0.02\%$  RSE) (29,30). These properties of TIMS are superior to inductively coupled plasma (quadrupole) mass spectrometry (ICPMS), which has a typical isotope ratio measurement precision of about 0.5% relative standard deviation for the major lead isotope ratios in blood (40,41). Thus, in many cases ICPMS may not reliably distinguish sample isotopic compositions that differ by less than 1%, as occurred between the paired blood and bone samples in this study.

The method used to calculate the amount of lead in blood that was derived from the skeleton deserves additional discussion. As previously noted, the relative amount of skeletal lead (mean  $\pm$  SD) in the circulation was calculated using both the  $^{207}\text{Pb}/^{206}\text{Pb}$  and  $^{208}\text{Pb}/^{206}\text{Pb}$  ratios (Table 1). This may provide a better estimate of that value in cases where an ideal two-source system does not exist or when the isotopic composition of one of the lead sources is not well known.

For example, when the estimated isotopic composition of the current exogenous exposure nearly falls on the line defined by the blood–bone pair (e.g., subject 3, Fig. 3), then essentially the same value is obtained for the percent bone lead contribution to blood lead (72% versus 71%) by using the  $^{207}\text{Pb}/^{206}\text{Pb}$  versus the  $^{208}\text{Pb}/^{206}\text{Pb}$  ratios. However, different results for the bone lead input to blood are obtained by using the  $^{207}\text{Pb}/^{206}\text{Pb}$  versus the  $^{208}\text{Pb}/^{206}\text{Pb}$  ratios when the estimated isotopic composition of the current exposure is a poorer approximation of the net exogenous exposure for a subject. This latter example is the case for subjects 1 and 2. This is also illustrated in Figure 3, where subject 1's bone lead contribution to blood was calculated to be 55% and 67% based on the  $^{207}\text{Pb}/^{206}\text{Pb}$  and  $^{208}\text{Pb}/^{206}\text{Pb}$  ratios, respectively.

However, using both the  $^{207}\text{Pb}/^{206}\text{Pb}$  and  $^{208}\text{Pb}/^{206}\text{Pb}$  ratios may not always provide a better estimate of the bone lead input. When the estimated lead isotopic composition of the current exogenous exposure falls on the blood–bone line, but at a point different from the “true” (unknown) exogenous exposure, using both ratios will yield the same result for the percent bone lead contribution to blood lead. However, the result will be biased by an amount proportional to the difference between the estimated current exogenous exposure and the “true” exposure.



**Figure 3.** Example of the method used for calculating the relative amount of lead in blood that was derived from bone. Included are the paired blood and bone samples from subjects 1 and 3, and their estimated current exogenous exposure (uncertainty oval omitted). Here, the estimated isotopic composition of the exogenous exposure appears to be a relatively good approximation of that exposure for subject 3 (i.e., it nearly falls on the dashed line defined by his blood–bone pair). In contrast, the estimated exogenous exposure appears to be a relatively poor approximation of the exposure for subject 1, so that different results for the bone lead input to blood are obtained by using the  $^{207}\text{Pb}/^{206}\text{Pb}$  (point A) compared to the  $^{208}\text{Pb}/^{206}\text{Pb}$  (point B) ratios (see Discussion).

The isotopic composition of the current net exogenous exposure was qualitatively derived from the isotopic compositions of previously measured urban aerosols/dusts and tap water samples from the San Francisco area (28,36) and from current exposure values extrapolated from the regression lines defined by the blood–bone pairs of subjects 1, 2, 3, and 4. This derivation of the current exogenous exposure is justified by the importance of urban aerosols/dusts as both a direct and indirect source of lead to humans and other organisms (14,28,35–37,42–45). It is also justified by assumptions inherent to the two-source mixing model. The lead sources and a sample mixture (i.e., blood) containing some amount of each lead source should fall along a common line when the data are expressed on a three-isotope plot (29,31). However, because this value for current exposure is a composite estimate, it may not exactly reflect the true current net exposure for each of the subjects that is indicated by the line extrapolated from their blood–bone pair (Fig. 3).

Although it is apparent that the lead contained in the skeletons of these subjects has been accumulated from exposures years or decades earlier, it is not possible to determine how much of their current skeletal lead burden was derived from past compared to more recent exposures. In other words, it is not possible to say how much “older” lead is contained in their skeletons. This is because little is known about the subjects’ past exposure history to lead in regard to its isotopic composition; there are relatively few data on the isotopic compositions of industrial lead emissions in the United States or elsewhere over past decades. In the case of subject 1 (Fig. 1), we used the data of Chow and Johnstone (25) as an example of the isotopic compositions of industrial lead that may have served as a source of exposure decades earlier. However, it is likely that the isotopic compositions of past lead exposures have been both spatially and temporally variable (14,27). Similar arguments can also be made for any of the other four subjects. Nonetheless, the skeletons of all the subjects contained  $^{207}\text{Pb}/^{206}\text{Pb}$  and  $^{208}\text{Pb}/^{206}\text{Pb}$  ratios that were larger than in their paired blood samples, indicating that the lead accumulated in their skeletons from past exposures was less radiogenic (i.e., lower relative amounts of the isotope  $^{206}\text{Pb}$ ) than the more recent exogenous exposures.

The subjects recruited for this study underwent total hip or knee arthroplasty due to degenerative joint disease/osteoarthritis of the hip or knee joint. The influence of this pathology on the lead content of the

analyzed bone specimens or on the release of lead from bone are not known. It has been suggested that the amount of lead released from bone back into the circulation may be increased during periods of increased bone turnover and/or net resorption, such as may occur during physiologic stresses (e.g., pregnancy, lactation, dietary mineral deficiency) and disease (e.g., hyperparathyroidism) (17–21,46). Therefore, the large amount of bone-derived lead contained in the circulation of these subjects might reflect a relative increase in bone lead release due to degenerative joint disease/osteoarthritis.

The health risks associated with lead released from the skeleton are not known. In children, toxic effects of lead on the central nervous system have been shown to occur at relatively low exposures (47–50), and there is still no established lower threshold for lead toxicity (48,51,52). Unfortunately, no similar data have been produced for populations prone to increased bone mineral loss, such as the elderly.

This stable lead isotope methodology is qualified by the confounding effects of isotopic variability and heterogeneity in the exogenous and endogenous sources of lead exposure. As illustrated in Figure 3, uncertainty or variability in the isotopic composition of a lead source (e.g., current exogenous lead exposure) increases the uncertainty in the calculated relative proportion of that source contained in the sample mixture (blood). Isotopic heterogeneity within the skeleton (either intra-trabecular or between trabecular and cortical bone) may also contribute to a biased assumption of the isotopic composition of lead in bone when only a representative sample is measured. This bias may introduce error in the calculated bone lead input to blood, resulting in an over- or underestimate of that value depending on the effective isotopic composition of the sampled bone compared to the skeleton as a whole and any relative difference in the rate of bone lead release between the measured bone and the whole skeleton.

The potential magnitude of this bias can be illustrated using the data of subject 1. If cortical bone contains  $^{207}\text{Pb}/^{206}\text{Pb}$  and  $^{208}\text{Pb}/^{206}\text{Pb}$  ratios that are approximately 1% larger than the ratios of 0.8550 and 2.082 measured in trabecular bone (reflecting a greater proportion of “older” lead contained in cortical bone) (27), and trabecular and cortical bone contributed equally to the lead in circulation, then the calculated bone lead input to blood for subject 1 would be 49% rather than the 61% reported here. This may be an overestimate of the potential bias, since trabecu-

lar bone may contribute more lead to the circulation than cortical bone under steady-state conditions, based on the 2- to 5-fold greater blood perfusion and turnover rates of trabecular versus cortical bone (6,53,54).

There may be ambiguity concerning bone as an endogenous source of lead to the circulation. This ambiguity arises largely from the complex nature of bone physiology and lead kinetics within the skeleton. Although bone functions as a long-term storage site for lead, there is also believed to be short-term (<days) exchange of lead between bone and the circulation associated with bone surfaces, intermediate (<months) exchange associated with the bone volume, and long-term (years) exchange associated with diffusion and bone turnover (apposition/resorption) processes (1,2). It is not possible to identify the relative contribution of these specific "compartments" or processes to the release of lead from bone using the stable isotope methodology as applied here. Rather, these data likely reflect the net physiologic process contributing to bone lead release.

## Summary

This initial study demonstrates the utility and limitations of the stable isotope methodology for evaluating the release of lead from bone in humans. The skeleton is an important endogenous source of lead exposure in environmentally exposed subjects. However, the small number and medical condition (degenerative joint disease/osteoarthritis) of the subjects presented here may limit the extrapolation of these results to the general population. More extensive studies with a larger cohort are in progress to better identify the isotopic signature of exogenous exposures, as well as the spatial heterogeneity in bone lead isotopic compositions within individuals. Those data will allow us to evaluate with greater accuracy and certainty the role of the skeleton as a source of lead exposure in humans.

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## Health Effects of Boron

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This issue of Environmental Health Perspectives Supplements, volume 102, number 7, includes papers presented at the International Symposium on Health Effects of Boron and Its Compounds, held September 16-17, 1992, at the University of California, Irvine. Borates and boric acid, which through gross medical misuse years ago gained a reputation for acute poisonings and fatalities, have in recent years received growing attention from two separate, major groups of investigators. One group has been pursuing evidence that boron is an essential element to humans, with a regulatory role in calcium metabolism and energy substrate use. The other group has been studying the reproductive and developmental toxicity of boron and the borates and has found boric acid at high doses to be a model compound for the study of mechanisms of reproductive and developmental toxicity.

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